Genetic and Environmental Effects on Corn Stover Composition
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Abstract

The chemical composition of corn stover can vary substantially, and this variability can significantly affect processing yields and economics. The primary aim of this work is to characterize the extent of variability in corn stover composition and assess its effect on biomass conversion process economics. A secondary objective is to try to understand the main causes of compositional variation in corn stover, particularly the extent to which variation is caused by genetic and/or environmental factors.

The number and variety of corn stover samples evaluated in FY02 was greatly expanded relative to previous work. Samples from over one hundred genetically distinct commercial corn stover varieties were collected from sites in 10 states (Iowa, Wisconsin, Indiana, Illinois, Minnesota, Ohio, Nebraska, South Dakota, Michigan and Tennessee). Many hybrids were collected from more than one site. In total, over 1100 stover samples from the 2001 crop were obtained in FY02, and the chemical composition of over 700 of them was determined using a robust calibrated near-infrared (NIR) spectroscopic method (Hames, 2002). Basic statistical methods were employed to characterize the population of samples surveyed.

Compositional data were evaluated using the Biofuels Program's ASPEN+ bioethanol process model (Aden, et al, 2002) to estimate the impact of compositional variability in raw stover materials on process economics. The range of carbohydrate content found in corn stover is surprisingly wide and indicates that feedstock composition variability can have a very large impact on process economics. Specifically, the structural carbohydrate content among 738 corn stover samples analyzed varies from 45.3 - 68.5%, resulting in a Minimum Ethanol Selling Price (MESP) ranging from $1.04 – 1.36/gallon ethanol.

In the near-term, it is recommended that efforts be continued in the area of monitoring corn stover quality across a wide range of genetic and environmental factors. In addition to monitoring commercial hybrids, a set of more exotic varieties (not commercial hybrids) already collected should be surveyed to determine whether or not the wider range of genetic diversity represented by these samples further extends the range of compositional variation in corn stover. This knowledge may provide useful tools in future breeding programs to optimize corn for dual use in producing grain and biomass feedstock. Efforts should also be made to cultivate working relationships with corn breeders and agronomists at public and private institutions to enlist their support in developing varieties and agronomic practices that improve biomass process economics through management of corn stover composition.

It is also suggested that longer-term efforts be initiated to pinpoint the major genetic and environmental factors that influence corn stover quality. This effort should be conducted in partnership with the USDA and could possibly leverage off of on-going efforts in the area of forage quality. The involvement of corn breeders and agronomists at universities and seed companies should also be considered. Knowledge of the factors that influence corn stover quality will suggest testable methods to manage corn stover quality.

Differences in processing efficiencies between different lots of corn stover are real and the basis for these differences need to be better understood. One possible explanation for differential processing efficiency is that cell wall architecture is not uniform. We currently have no tools to determine if this might be the case. Therefore, a second longer-term effort is suggested to develop tools and methods for detecting differences in plant cell wall architecture.

I. Introduction

During the last 3 years, the Biomass Program has become acutely aware of the fact that the chemical composition of corn stover (i.e., stover quality) can vary substantially, and that this variability can have major effects on processing yields and efficiencies, and therefore process economics. The main goal of this project is to assess the extent to which commercial hybrid corn stover quality (i.e., chemical composition) varies, and how that variation influences biomass conversion process economics.

When the Project started working with corn stover in FY00, it was not appreciated that corn stover composition would be a major variable in biomass processing. Initial indications of feedstock variability arose during chemical analysis of corn stover for pretreatment experiments. As a result, in FY01 the program initiated an effort to assess the range of variability in chemical composition of corn stover. During FY01 the range of compositional variation among 18 hybrid corn varieties grown at one of two locations was assessed and the effects on process economics were documented in a C milestone report (Thomas, et al., 2001). The main conclusions from that preliminary work are listed below.

- An 8% span of glucan plus xylan content was observed among the 18 samples.
- This range of carbohydrate content translates to a range of minimum ethanol selling price (MESP) of 20 cents/gallon of ethanol.
- Different anatomical fractions of corn stover from the same plants have different compositions. It is therefore important to consider harvesting, baling, transport and storage logistics as factors that could bias what parts of the plants are input to the conversion process.
- Differences in pretreatment yield were shown, in one case, to translate into a significant cost savings (~10 cents/gallon of ethanol) to the process. These results support the possibility that stover cell walls differ not only in composition, but that they may also be architecturally distinct, such that thermochemical pretreatment processes may exhibit differential performance under similar processing conditions.

Due to the limited number of samples analyzed in FY01, we suspected that stover composition might range even more widely. Therefore, during FY02 we undertook to sample a much broader cross-section of commercial corn stover. We specifically sought to include wide diversity among samples in terms of both genetic and environmental factors. We have done this in an attempt to get a better sense of the nationwide variability present in stover from the 2001 crop, as well as to develop a preliminary sense of the potential causes of this variability. This information will be helpful in designing a versatile and cost-effective corn stover-based process and will provide valuable experience and insight that can be employed with current and future development partners.

II. Compositional variability among raw corn stover samples

A. Descriptions of sample sets

Each year dozens of seed companies market a bewildering array of hundreds of hybrid corn varieties to farmers in the United States. The selection of varieties offered varies from year to year as some varieties become obsolete and new varieties are released onto the market. Somehow, farmers must assimilate all this information and make informed decisions about which varieties to grow in their particular farming situation. To assist in this process, extensive field trials are performed by seed companies, as well as independent entities, to determine the environments where each variety can be expected to perform well. Hybrid performance is generally understood to mean the highest yielding (grain) varieties in today's market. Stover quality is not an issue in grain trials except as it relates to stalk lodging and the effect that has on grain yield. Seed companies and many Corn Belt university agronomy departments freely provide information about hybrid performance to farmers. This information greatly decreases the number of hybrids that need be considered by a particular farmer and greatly assists him/her in making decisions about which hybrid(s) to purchase.

Two general categories of factors are expected to influence the compositional variability observed in corn stover – genetics and environment. The precise combination of approximately 50,000 genes in the corn genome defines the basic physical and agronomic characteristics of a particular hybrid. Different combinations of genes are brought together by the presumably unique parentage of each hybrid. As a result different hybrids have different growth and performance characteristics. Because the genetic lineage of each of each corn hybrid is usually a tightly held trade secret, it is usually not possible to ascertain how closely related any two hybrids are. Since one of our goals is to maximize genetic diversity among samples in this study, our approach has been to collect stover from a range of different hybrids from as many different seed companies as possible.

In addition to genetics, environmental factors are also important variables in crop performance and it would not be surprising to learn that environmental factors can influence stover quality. The category of environmental influences includes all variables that the plant experiences during its life cycle that are related to location (e.g., climate, day length, soil type) and local agronomic practice (e.g., tillage method, irrigation, fertilizer type and amount, agricultural chemical use, harvesting practices). Some environmental factors can also be expected to interact with genetic variables. In other words, environmental factors may modify genetic potential.

As a result of these considerations, we decided to pursue a set of stover samples for this study that are as geographically and genetically diverse as possible. Over 1100 stover samples grown during the summer of 2001 were acquired from a variety of sources in FY02. Corn stover suppliers and the number of samples provided by them for this work are listed in Table 1. In most cases, corn stover samples were obtained under a standard purchase order agreement but some of the samples were generously donated.

Separate discussions were held with each stover supplier to identify appropriate sample sets for this work. Each supplier was asked to try to maximize genetic diversity from among the varieties they were growing, but for the most part even these experienced people are not well informed regarding the degree of relatedness among corn hybrids. Once the sample sets were identified, suppliers were asked to provide 5-10 pound (10-20 whole stalks, minus cobs) for each sample. Since individual suppliers probably employed slightly different sampling strategies, it is likely that the proportions of leaves, stalks and cobs vary among the groups of samples. As we did not attempt to specify the harvesting method used by our suppliers, or the timing of stover harvest, we had no control over the anatomical representation of the samples collected. For instance, in some cases, the grain was mechanically harvested from the field before collection of the stover. In other cases, the grain may have been harvested by hand (a much gentler process). Harvesting of grain using a mechanized combine cracks and breaks the stalks and shatters the dried leaves as the tractor moves through the field. Hand harvesting most likely better preserves the anatomical integrity of the stover. If the stalks in a mechanically harvested field experienced rain between the grain and stover harvest times, a large fraction of any water-soluble solids present in the stover may have been washed out of the sample. Differences in the timing of stover harvest relative to the grain harvest, as well as the harvest method used and amount of precipitation between grain and stover harvests should be considered as environmental factors in assessing the potential causes of variability in stover composition. No attempt was made in this work to try to quantitate differences in relative proportions of anatomical fractions.

Table 1 – Sources of 2001 Corn Stover

Source	Contacts	# Samples
Monsanto Corp.	Diane Freeman, Brad Krohn, Dale Sorensen and others	217
University of Minnesota	Dale Hicks, Tom Hoverstad and Steve Quiring	150
University of Wisconsin, Madison	Joe Lauer (and Jim Coors)	205
USDA/ARS, Lincoln, NE	Wally Wilhelm	96
University of Nebraska, Lincoln	Ken Russell	63
Iowa State University	Wayne King	~30
Iowa State University and USDA/ARS, Ames, IA	Linda Pollak	45
USDA/ARS, North Central Plant Introduction Station, Ames, IA	Mark Millard	245
ProdiGene, Inc.	Donna Delaney (and Beth Hood)	14
University of Tennessee	Chad Eden and Lester Pordesimo	80
University of Kentucky	Mike Montross	??
Total		**>1145**

Most of the samples collected come from commercial hybrid varieties marketed by many seed companies to farmers in the U.S. However, some of the samples collected are from obsolete hybrids, inbred lines, introgressed lines, exotic accessions or ancestral landraces. For the most part, these latter types will not be included in this report, as resources did not permit analysis of most of those samples. Due to limited resources for this project, it was necessary to prioritize the samples to be processed during the year (see Table 2). Commercial hybrid varieties grown in three or more locations (or cultivation regimes) were assigned highest priority. Commercial hybrids grown in less than three locations (or cultivation regimes) were assigned the next highest priority. A few inbred varieties and exotics were also included in the analysis. For the most part, only samples in the shaded rows of Table 2 will be discussed in this report.

The University of Minnesota and University of Wisconsin (as well as other corn belt states) conduct annual side-by-side comparative grain yield trials for corn seed producers who market seed in those states. The purpose of these trials is to provide unbiased and trustworthy information to farmers who must make decisions about which corn varieties to plant in their fields the following spring. Since the stover from these trials is regarded as a waste product at the end of the yield trial experiment, we were able to acquire stover samples from a wide variety of genetically distinct varieties that were grown in more than one environment by the same organization.

Table 2 – Prioritized Sample Processing List

Priority	Source	Samples	Hybrids	Inbreds	Other Types	Environments	Treatments
1	Monsanto Corp.	217	21	0	0	38	no
2	Univ. Minnesota	150	50	0	0	3	no
3	Univ. Wisconsin	205	31	0	0	2 - 6	no
4	ARS, Lincoln	96	9	0	0	1	Irrigation, fertilization
5	Univ. Nebraska	63	some	some	some	1	Irrigation, fertilization, planting density
6	ARS, Ames	45	0	0	all	1	no
7	ARS, NCRPIS	245	some	some	most	1	no
8	Iowa State Univ.	~30	all	0	0	several	different farmers
9	ProdiGene, Inc.	14	0	14	0	1	no
10	Univ. Tennessee	80	2	0	0	1	harvest time, anatomical fractions
11	Univ. Kentucky	??		0	0	1	Post-harvest storage

The University of Minnesota provided 150 5-10 pound samples of stover from 52 commercial varieties grown for grain yield trials at each of three locations in Minnesota – Lamberton, Waseca and Plainsview/Potsdam. These locations span the southern zone of the state from east to west and are marked on the map in Figure 1. Samples were thoroughly dried prior to shipping to NREL. Both early and late maturing varieties were planted at each site. Only a subset of the varieties planted in the Minnesota trials was sampled for this study. Branded hybrids sampled in this study come from Asgrow, Brown, Dahlman, Dairyland Stealth, Dekalb, Epley Brothers, Garst/AgriPro, Jung, Kruger, NC+ Hybrids, Northrup King (Syngenta), Pioneer Hi-Bred, Ramy, Viking and Wilson. A more detailed description and the results of the "2001 Minnesota Hybrid Corn Performance Trials" can be found online (Hoverstad, et al, 2001).

The University of Wisconsin provided 205 5-10 pound samples of stover from 31 commercial varieties. Samples were obtained from 8 locations in Wisconsin, representing the southern, south central and north central zones of the state. These locations are marked on the map in Figure 1. Arlington, Janesville and Lancaster are located in the southern zone. Fond du Lac, Galesville and Hancock (irrigated) are located in the south central zone. Chippewa Falls and Seymour are located in the north central zone. Hybrids were replicated at all locations in the same zone and were occasionally planted in two zones, but never in three. Hybrids were not replicated at all locations, as they are typically grown only in favorable environments for that variety. Samples harvested were thoroughly dried prior to shipping. Branded hybrids sampled in this study come from Asgrow, Cargill, Dairyland Stealth, Dekalb, Garst/AgriPro, Midwest, Northrup King (Syngenta), Pioneer Hi-Bred and Wyffels. A detailed description and the results of the "2001 Wisconsin Corn Hybrid Performance Trials for Grain and Silage" can be found online (Lauer, et al, 2001).

The 217 Monsanto samples were provided at no cost to the Biomass Program through the generosity and cooperation of Brad Krohn, Dale Sorensen, Diane Freeman, and a host of others at Monsanto's various field stations. Samples were provided with coded identifications (i.e., "blind" samples) from 21 commercial hybrids (presumably representing Dekalb and Asgrow brands) grown at 38 different sites throughout the U.S. Corn Belt (see Figure 1). Hybrids were not replicated at all locations, as they are typically grown only in favorable environments for that variety. All of the Monsanto samples were sent to NREL at Monsanto's expense via 2nd Day UPS shipping prior to drying. Every effort was made to dry them immediately after arrival.

Figure 1 – 2001 Corn Stover Collection Locations

Figure 1. Map showing approximate locations where corn stover samples used in this study were produced. The three University of Minnesota locations are marked in dark blue. Eight University of Wisconsin sites are marked in shades of purple (north central zone sites are marked in light purple; south central zone sites are marked in medium purple; southern zone sites are marked in dark purple). Thirty-eight Monsanto test fields are marked in red. The University of Nebraska location is marked in light blue. The USDA/ARS, Lincoln site (Shelton, NE) is marked in bright green. The ProdiGene location (Aurora, NE) is marked in black. The ARS/NCRPIS location (Ames, IA) is marked in dark green. Iowa State University locations (2) are marked in blue. The University of Tennessee location is marked in pink.

Dr. Wally Wilhelm generously provided the 96 USDA/ARS (Lincoln, NE) stover samples for the cost of shipping them to NREL. Samples were grown at Shelton, NE, and thoroughly dried prior to shipping. NREL was provided with three replicate samples of each of 8 hybrids for each of 4 treatments. The four treatments were comprised of all combinations of plus and minus nitrogen fertilizer coupled with plus and minus irrigation. Seven of the eight hybrids were Pioneer varieties, while the last was a public hybrid (B73 x Mo17). All plot replications were carried out at the same location, so differences in soil composition and agricultural practice can be discounted in the analysis of these samples. Since a few of the samples were lost or mislabeled, only 5 hybrids were represented by triplicate samples, and these were used in the analysis of variance.

The 63 University of Nebraska samples were harvested after a carefully designed study to look at the effects of irrigation and planting density on grain yield, and were provided by Dr. Ken Russell. Some of the samples are from commercial and other hybrids, while others are from inbreds, breeding populations, ancient landraces and a related species (i.e., teosinte). The commercial hybrid used in this study was a

Pioneer variety (33P67), and it was again compared to the public hybrid, B73 x Mo17. Replicate samples were collected and thoroughly dried prior to shipping.

Dr. Donna Delaney (ProdiGene, Inc.) kindly donated stover samples from 14 inbred lines grown at their field test site near Aurora, NE, during the summer of 2001. Samples were not dried prior to shipping (UPS ground) and arrived at NREL with mild contamination from mold growing on them, especially around the stalk nodes. Samples were dried immediately upon arrival at NREL.

Dr. Lester Pordesimo and his student, Chad Edens, of the University of Tennessee, provided samples harvested at different times after planting from two Pioneer varieties grown near Knoxville, Tennessee. Four interior plots in the field, one planted to Pioneer 32K64 (a genetically engineered Bt hybrid) and another to Pioneer 32K61 (the conventionally bred parental line to 33K64), were selected for sampling. Except for a single gene encoding a *Bacillus thuringiensis* (Bt) toxin, these two hybrids are considered genetically identical. This study was designed to see how the composition of anatomical fractions of plants changes as a function of maturity during the latter part of the growing season. Sampling began at the late dent stage of seed filling and continued until four weeks after the time the grain was deemed suitable for harvest. Grain was judged ready for harvest in mid-September and some plots not immediately bordering the sampled plots were harvested on September 28, 2001 using a two-row field plot combine.

Roughly twice a week, between August 9 and November 26, 2001, two randomly selected corn plants were manually harvested from each experimental plot. The plants were cut 15.2 cm (6 in.) from the ground and taken to the laboratory for processing. The cut plants were carefully separated into leaves (leaf blades only), stalks (including tassel and leaf sheaths), husks (including the cob shank), and ears (minus grain). The sheath resembled the tougher stalk material and was it was therefore decided to include it as part of the stalk fraction. Samples were frozen at -20^{o}C until it was convenient to dry and mill them. Samples were dried and milled and then shipped to NREL for compositional analysis. These samples will provide a better understanding of how stover quality changes as a function of plant anatomy late in the life cycle of the corn plant, and will provide some insight in to the fate of "soluble solids" in corn stover.

B. Sample preparation and compositional analysis methods

Samples were delivered from suppliers almost continuously from October, 2001, through January, 2002. Each of the 1100 stover samples was logged into NREL lab notebooks, given a unique NREL identification number, and photographed. Samples that arrived wet were dried for several days in a warm, dry greenhouse to a relatively low moisture content (i.e., until they were not in danger of biological degradation). Samples were then thoroughly dried by a local subcontractor in a circulating oven at 50^{o}C for 2-3 days. The subcontractor employed a yard waste shredder to coarsely shed each sample separately. Shredded samples were then milled with a rotary knife mill to pass a 0.25-inch screen. Samples larger than 500 grams total were riffle-split to ensure that different aliquots of the sample were as equivalent as possible. Samples were then packaged in labeled, 1-gallon Ziploc bags (500 gram aliquots) inside labeled plastic buckets. Uniformly milled samples were analyzed to determine the composition of each.

The rapid analysis near-infrared (NIR) spectroscopic method for determination of corn stover composition has been previously described in Ethanol Project milestone reports (Hames, et al, 2000; Hames, 2002), at Stage Gate review meetings, and has been submitted for publication. The method itself will not be further discussed here, except to point out that it is constantly undergoing improvement, as new samples are acquired that expand the calibration range of the NIR model. All NIR compositional information presented in this milestone report were produced using the 'Stover5c' version of the corn stover model (Hames, 2002).

C. Analysis of Variance

Analysis of variance (ANOVA) was performed using the data analysis add-in tools provided with Microsoft Excel. Specifically, we used the "ANOVA: Two Factor with Replication" tool. The value of alpha was set at 0.05. Triplicate samples for each of 5 hybrids and 4 cultivation treatments included in the USDA (Lincoln, NE) experiment were included in the analysis.

D. Results

1. Analysis of whole stover samples (n=738)

Output from the 'Stover5c' NIR model produces data for the following components of corn stover: total glucan (includes glucose in cell wall structural polymers as well as soluble sugars, such as sucrose), structural glucan, xylan, arabinan, galactan, mannan, uronic acids, lignin, protein, acetyl, structural inorganics , and soil. The compositions of all samples discussed in this report can be obtained on request from Steve Thomas (steven_thomas@nrel.gov). The 738 compositions discussed below do not include the University of Tennessee samples, as they apply to different anatomical fractions of plants and will be treated separately.

Of the 738 stover samples whose composition was determined, 668 of them are from commercial hybrids. An additional 29 samples are from a single public hybrid. Thus, this work is highly relevant to the nascent biomass processing industry. The database of stover compositions includes samples from 112 different commercial hybrids representing 22 brands that were grown at a total of 52 locations in the Corn Belt. A subset of the hybrids selected for this work were grown at more than one location in an attempt to get a preliminary sense for the extent to which environmental factors can influence stover composition.

In the NIR analysis of samples only 8 of the 738 samples (1.1%) produce a global-H value greater than 3.0, which is used as a statistical flag for reliability of the data produced by the model. This rather remarkable statistic shows that the Stover5c rapid analysis method is capable of producing reliable data from ~99% of the samples presented to it, which is a powerful testimony to the robustness of this technique.

A set of summary statistics for this population of samples is presented in Tables 3 and 4. It is readily apparent that there is substantial variability in the composition of commercial corn stover materials collected. Depending on assumptions made about which sugars can be fermented (i.e., glucan plus xylan vs. all five sugars), the fermentable sugar content present in structural components of stover cell walls spans a range between 19 – 23 weight percent. It is important to realize that this conclusion specifically *excludes* any soluble sugars that may be present in the material. Soluble sugars are treated as non-fermentable, based on the assumption that they are degraded during dilute acid pretreatment. It should also be noted that the range of structural sugar content has been significantly widened relative to the 8% (glucan plus xylan only) reported in FY01 (Thomas, et al, 2001).

Once the composition of all the samples was determined, it was of interest to know how the values for each constituent are distributed around the mean value. Figure 2 illustrates the distribution of values for eight constituents of corn stover biomass in the sampled population. While the distributions of the major constituents (i.e., structural glucan, xylan and lignin) approximate a normal distribution, some of the minor constituents (i.e., protein, acetyl, structural inorganics and calculated soluble sugars) may not. The reasons for this are not clear, but may be partially due to the magnitude of the error in the measurement as compared to the constituent values and ranges.

Since many samples are clustered around the mean value for each constituent we were curious to know what fraction of the samples from this large data set have a composition described by the set of mean values listed in Table 3. The method used to determine this follows. The upper and lower limits for one standard deviation (for example) around the mean value were determined for each of the 5 major constituents of corn stover: structural glucan, xylan, lignin, protein, and structural inorganics. The entire database was then sorted by increasing value for one constituent, and samples outside the one standard deviation range were eliminated from the population. The process was then repeated for each of the other constituents, in succession. Only 140 (19%) of the original 738 samples remained after this culling process. If more stringent restrictions are applied to this approach (i.e., +/- 5% variation from the mean value), there are zero samples that survive the culling process. This means that not a single sample in the database can be described by the set of mean values listed in Table 3.

Table 3 – Summary Statistics for Individual Constituents of Corn Stover

	total_glucan	struct_glucan	xylan	lignin	protein	acetyl	uronic_acids	galactan	arabinan	mannan	st_inorg	soil	Calculated Soluble sugars	Total
Minimum	34.46	27.86	14.49	11.54	1.27	0.86	1.40	-0.44	-1.15	0.05	-1.15	0.86	2.01	89.99
Maximum	50.26	39.55	25.45	20.43	6.98	3.88	3.88	2.35	3.67	1.72	10.18	1.66	19.55	101.92
Range	15.80	11.69	10.96	8.89	5.70	3.02	2.48	2.79	4.82	1.67	11.33	0.80	17.54	11.93
Mean	41.95	33.79	19.96	15.82	3.61	2.69	2.87	1.59	2.50	0.84	4.22	1.33	8.16	97.38
Stdev	1.54	1.98	1.64	1.44	0.71	0.46	0.33	0.33	0.49	0.27	1.56	0.11	2.18	1.77
Count	738	738	738	738	738	738	738	738	738	738	738	738	738	738

Table 4 – Summary Statistics for Combined Carbohydrate Constituents

Statistic	Struc_glucan + Xylan	Struc_glucan + Xylan + Arabinan + Galactan + Mannan
Minumum	44.30	45.29
Maximum	63.26	68.52
Range	18.96	23.23
Mean	53.75	58.68
Std. Dev.	2.80	3.23
Count	738	738

Since one standard deviation includes 66% of a normally distributed population, five independent variables tracked for the same population will yield 0.66^5 (0.125) of the population lying within one standard deviation of the mean for all five variables. This means that the fraction of samples remaining after our culling process (0.19) is only about 50% greater than would be expected by random chance (0.125). We conclude from this that the weight fractions of the 5 major components of biomass are fairly independent of one another.

The observation that no samples resembling what might be interpreted as an "average stover composition" in Table 3 exist in our extensive database is somewhat surprising. It suggests that the "average corn stover composition" as used in NREL's process modeling, for instance, may not exist and may be very difficult to produce as a specific mixture of two or more lots of corn stover. It does not make sense to model a process (e.g., Aden, et al, 2002) on the basis of a hypothetical feedstock composition that is either extremely rare, or may not exist. The Biofuels Program should probably reconsider its choice of the feedstock basis for the process economic analyses. Either a real stover composition should be used, or it may even be worthwhile to express compositions as ranges (+/- one standard deviation?) rather than as specific values. It should also be noted that any future attempt at calculating an average composition for corn stover should also be weighted for hybrid market share in a particular region of interest. This information may not be easy to obtain.

We next asked whether there are any correlations between pairs of constituents in this population of samples. Scatter plots of selected pairs of constituents are presented in Figure 3 and correlation coefficients for the linear regression fits between all pairs of major constituents are listed in Table 5. Graphs in Figure 3 with stronger correlations have shaded backgrounds, while those with insignificant correlations have white backgrounds.

Table 5 – Correlation Coefficients (R^2) for Linear Regression Fits

	Struc_glucan	**Xylan**	**Lignin**	**Protein**	**Acetyl**	**Str_Inorg**
Xylan	0.04					
Lignin	**0.60**	0.16				
Protein	**0.70**	0.18	0.32			
Acetyl	0.19	0.02	0.15	0.10		
Str_Inorg	0.10	**0.41**	0.32	0.13	0.12	
Calculated Soluble Solids	**0.53**	0.14	**0.57**	0.27	0.16	0.01

There is only one reasonably strong positive correlation in the data set – structural glucan with lignin (r^2=0.60). This observation may be telling us that as stover cell walls mature both structural glucan and lignin contents increase. There are also four reasonably strong to rather weak negative correlations, including structural glucan with protein (r^2=0.70), xylan with structural inorganics (r^2=0.41), structural glucan with calculated soluble sugars (r^2=0.53), and lignin with calculated soluble sugars (r^2=0.57). As structural glucan content of cell walls increases during maturation, protein content may remain fairly constant on a per cell basis, which may partially explain the negative correlation between these two constituents. In light of the above-mentioned positive correlation between structural glucan and lignin, the observation that there is apparently only a weak (r^2 = 0.32) negative correlation between lignin and protein is somewhat confusing. The weak negative correlation between xylan and structural inorganics has been noticed previously and is difficult to explain. It may be that structural inorganics (silica bodies?) can functionally compensate (at least partially) for xylan, and vice versa. The negative correlations between soluble sugars and both structural glucan and lignin may be an indication that as plants age and dry down, soluble sugars become incorporated into cell wall polymers. On the other hand, these last two correlations may be a trivial consequence of the fact that as soluble solids are washed out of mechanically harvested stover in the field, structural components must mathematically compensate in the mass balance. None of the above ideas have been tested, so they should be regarded as hypotheses.

Lastly, most pairs of constituents in stover show little or no evidence of positive or negative correlation with other constituents. In particular, it should be noted that variability in xylan shows no correlation with structural glucan, lignin, or acetyl groups. This lack of correlation means that these constituents are relatively independent of one another. This conclusion is corroborated by a different method, described above.

Figure 2 – Distributions of 8 Constituents Among Stover Samples (n=738)

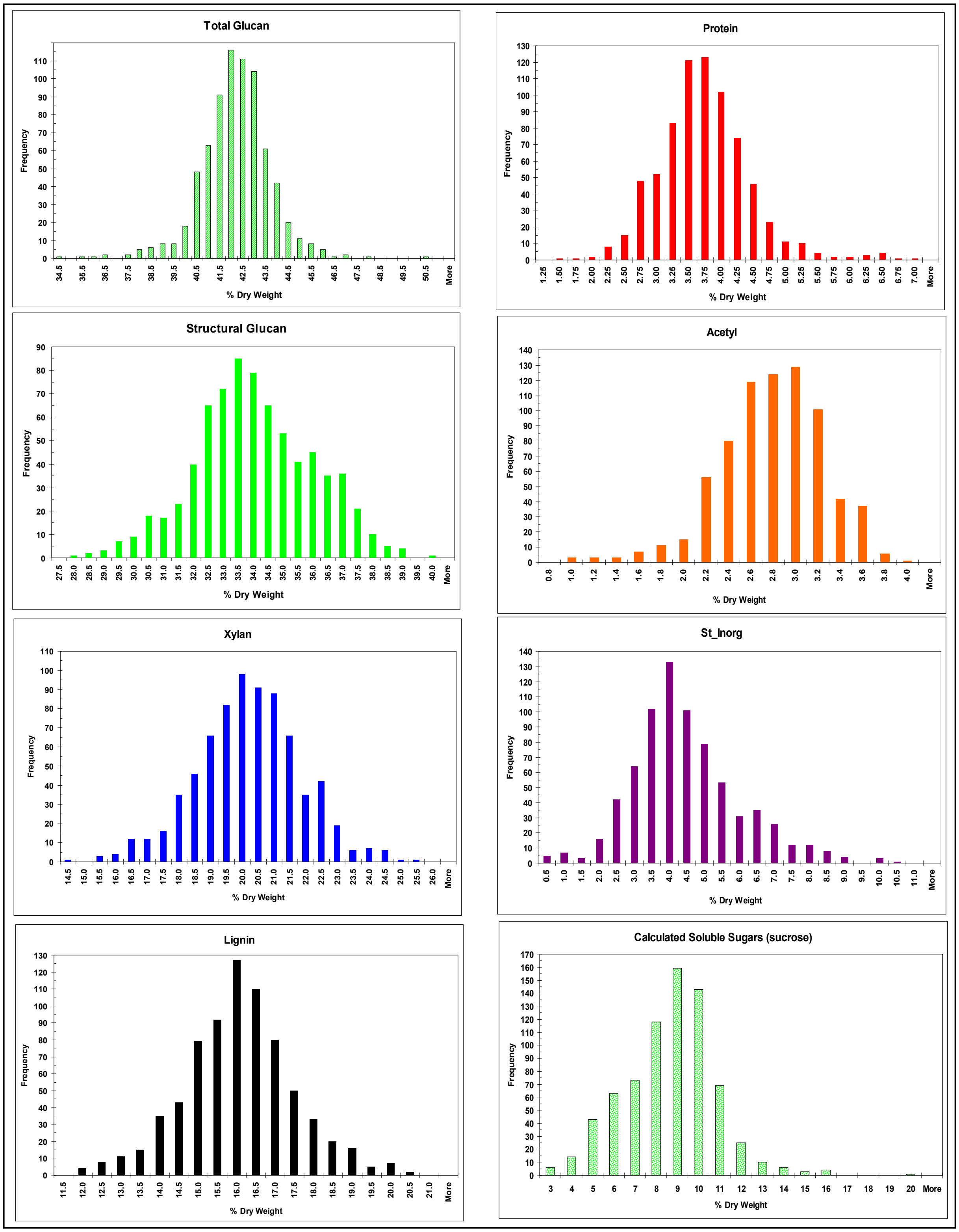
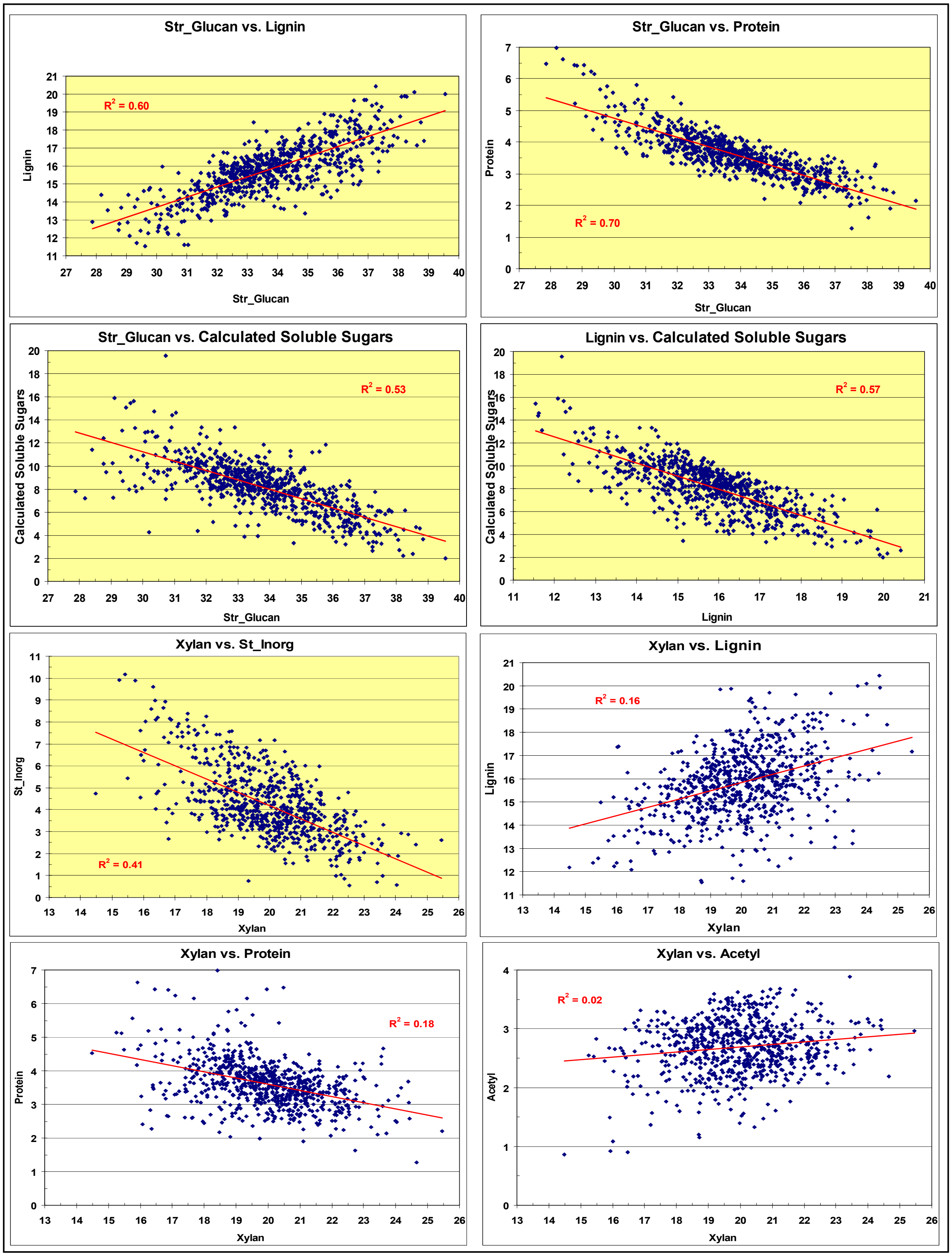


Figure 3 – Correlations Among Selected Corn Stover Constituents (n=738)



2. Effects of Genetics and Environment of Stover Quality

The replicated sample set provided by Dr. Wally Wilhelm at the USDA/ARS provides an opportunity to assess the effects of two environmental factors on a set of 5 hybrids that were collected from replicate plots in a full factorial design experiment carried out during the summer of 2001. Dr. Wilhelm was looking for effects of fertilization and irrigation on crop performance over a series of Pioneer hybrids and an older public hybrid called B73 x Mo17. Two fertilization levels (0 and 200 pounds/acre) and two rates of irrigation (zero and 3 inches/per week) were used in this experiment, thus providing 4 cultivation conditions. Of course, all plots profited by whatever natural precipitation happened to occur. The four Pioneer numbers used in this analysis are 3162, 3394, 33R88 and 34G82. Thus, there are 5 hybrids (genetic variable) grown in triplicate under four different environmental conditions at the same location (60 samples in total).

The compositional data from these samples were used in a statistical analysis of variance to obtain evidence supporting whether or not either of the two tested environmental variables, or genetic factors, or both, may act as causal agents for observed differences in composition. We looked at the response of structural glucan, xylan, lignin, protein and structural inorganics separately as a function of 5 genetic levels (hybrid name) and 4 cultivation conditions. Significance was determined at the $P=0.05$ level (i.e., 95% confidence that the means are not the same for all classes). While these results must be considered preliminary at this point, they do provide some interesting food for thought.

The results of the analysis of variance for structural glucan indicate that genetic ($P=1.06E-07$) and environmental ($P=1.27E-05$) factors are both highly significant. However, no significant interaction between the genetic and environmental factors was detected in the case of structural glucan ($P=0.17$). The same analysis was applied to xylan where it was found that genetics was not a significant factor ($P=0.15$), but that environmental conditions are ($P=5.81E-05$). A significant genetic by environment (GxE) interaction was detected ($P=0.003$) for xylan. Similarly for lignin, a significant effect of genetics was detected ($P=0.002$) while environmental effects were almost significant ($P=0.06$). The GxE interaction was not found to be significant for lignin ($P=0.11$). Variability in protein content was strongly influenced by both genetics ($P=1.46E-06$) and environment ($P=9.48E-11$), but no significant interaction between them was detected ($P=0.43$). Yet another pattern was detected for structural inorganics, where only the GxE interaction produced a significant result ($P=0.014$). We also looked at the sensitivity of the sum of structural glucan and xylan to genetic and environmental influences in this experiment. The pattern here is like that for structural glucan, where sensitivity to both genetic ($P=0.001$) and environmental ($P=0.002$) influences was detected, but there was no significant interaction between them.

The patterns of statistical significance detected in this experiment are presented diagrammatically in Table 6. An "x" in a cell indicates a statistically significant influence on variability for a particular constituent. It is not surprising that both genetic and environmental influences can impact stover composition. However, the conclusion that different constituents of biomass may respond differently to genetic and environmental factors is more difficult to explain. This area requires further investigation.

Table 6 – Factors Responsible for Compositional Variability in Corn Stover

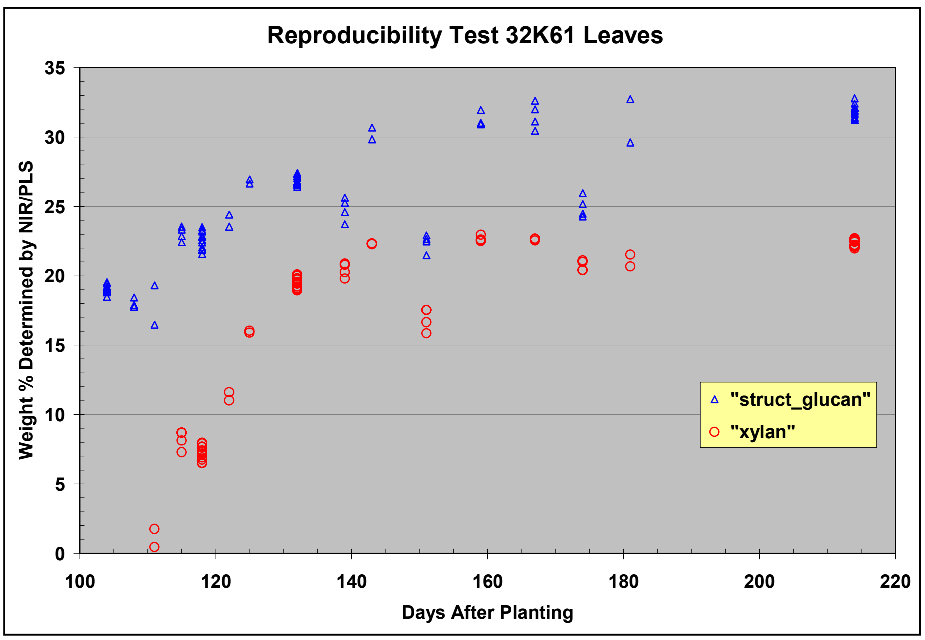
Stover Constituent	Genetics	Environment	Interaction (GxE)
Struc_glucan	x	x	
Xylan		x	x
Lignin	x		
Protein	x	x	
St_Inorg			x
Struc_glucan + Xylan	x	x	

3. University of Tennessee anatomical fractions

The samples provided by the University of Tennessee come from two Pioneer Hi-Bred varieties: 32K61 and 32K64. These hybrids are genetically identical, except that 32K64 is derived from 32K61 after being genetically engineered to insert one additional gene. The extra gene in the 32K64 genome encodes an insect toxin from the bacterium *Bacillus thuringiensis* (i.e., Bt toxin) that helps to control attack by insect pests (particularly the European corn borer). The intent of this study is to track the changes in chemical composition as a function of plant anatomy from prior to grain maturation to well after grain harvest to see if any changes in relative proportions of cell wall constituents occur.

Most of the samples in this study were subjected to NIR spectroscopy a number of times (i.e., up to 14). This provides a way to check the reliability of the method, including errors introduced due to sampling, different instrument operators, and the spectrometer itself. For the sake of clarity, only the data for structural glucan and xylan in 32K61 leaf blades are presented in Figure 4. However, all the constituents show this same quality of reproducibility from scan to scan. The data for each time point sample are tightly clustered in most cases, supporting the idea that the rapid analysis method for corn stover is quite robust and that the error associated with the method is approximately equal to those for the wet chemical methods.

Figure 4



Figures 5 and 6 compare the data from stalks for the two varieties, which were expected to be quite similar in composition since these varieties are so similar genetically, and were grown under essentially identical conditions. These data establish that the chemistry of similar tissues from these two varieties is essentially indistinguishable, and this pattern holds for leaf blades and ear husks, as well (data not shown). These observations are in contrast with the work of Saxena and Stotsky (2001), which claims to see a significant difference in lignin content between third internodes of stalks from of two similarly related Pioneer varieties.

Figure 5

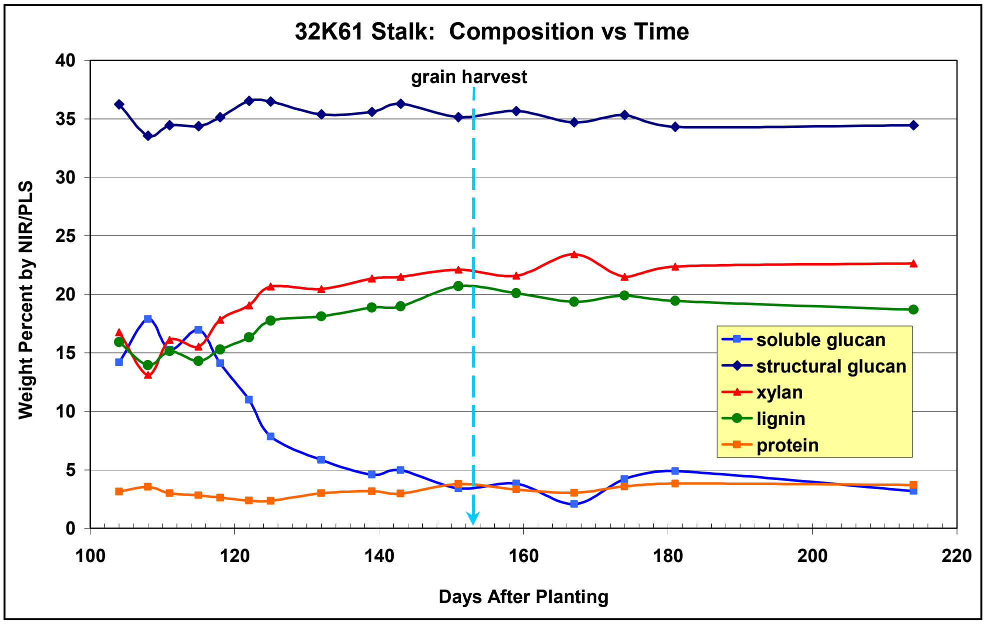
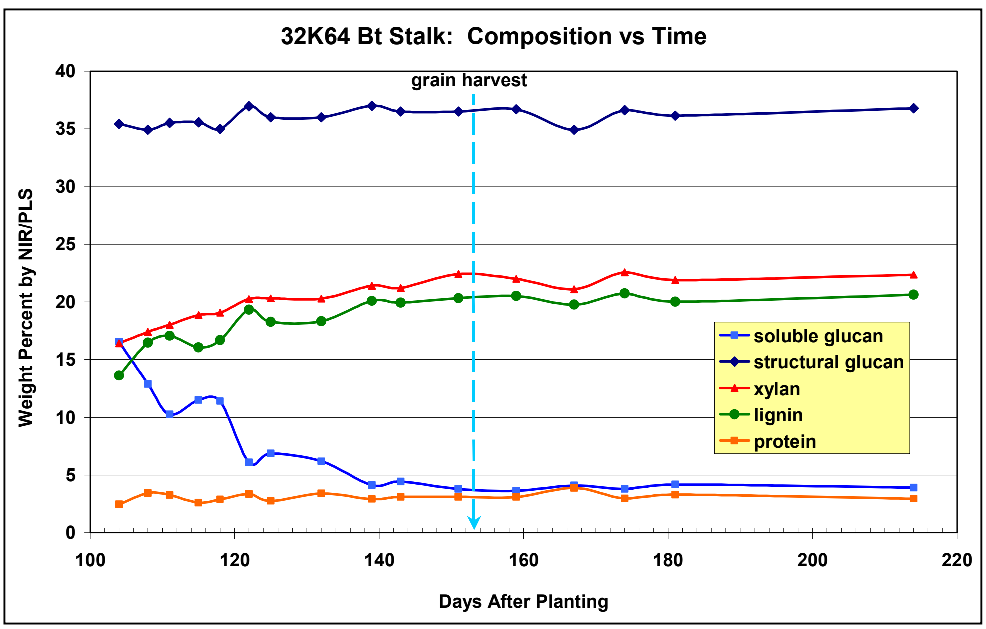


Figure 6



Figures 5, 7 and 8 show the composition of 32K61 stalks, leaf blades and ear husks, respectively, as a function of days after seed planting. The initial time point on these graphs (104 days) corresponds to the late dent stage of kernel development, and grain harvest in the surrounding plots took place in mid-September (day 153). It is interesting to note the progression of changes in chemistry that occur in these anatomical fractions as the crop matures. Both the leaf blades and ear husks are photosynthetic organs, so they fix carbon from the atmosphere and export the photosynthate (mostly to the grain while it is still filling). After grain maturity soluble glucan declines to a minimum in all tissues by day 151, about when the grain was harvested. After grain harvest the composition of the samples remains relatively constant.

Figure 7

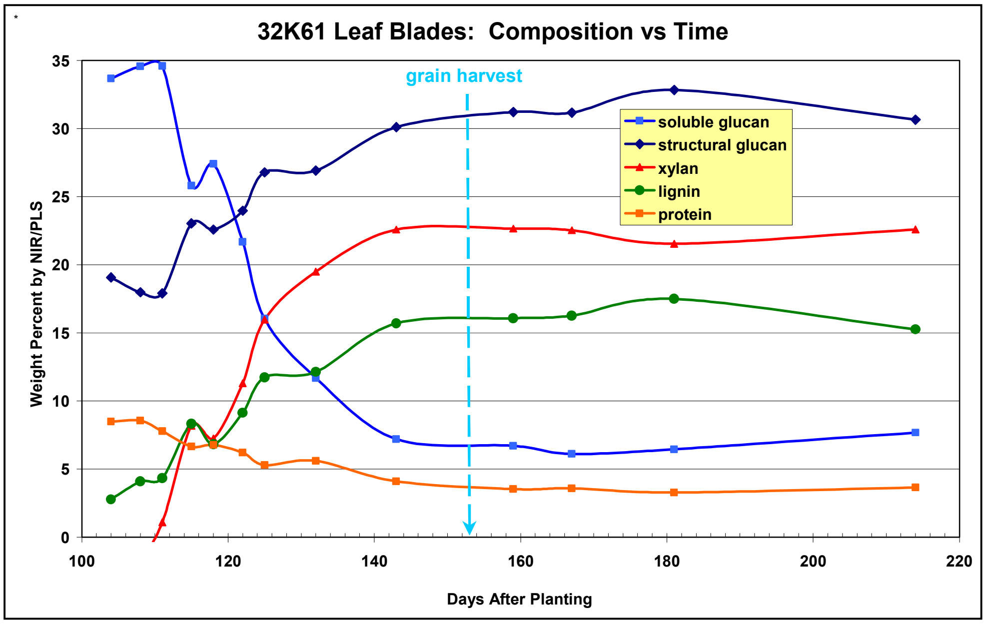
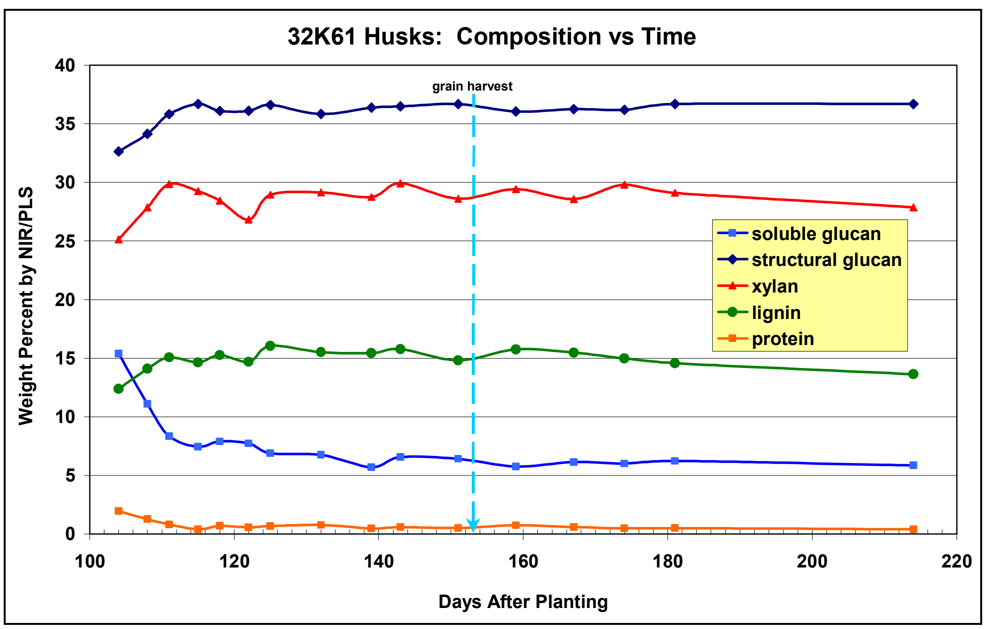


Figure 8



It is clear from Figures 5, 7 and 8 that the composition of these different tissues at full maturity (day 214) is distinct. While all three plant parts from 32K61 seem to have similar lignin content (14-15%), husks have a significantly higher xylan content (~28%) than either leaves or stalks (~23%), and leaves have a significantly lower cellulose content (~31%) than either stalks (~35%) or husks (~37%). Among these three anatomical fractions husks seem to have the highest polysaccharide content, though they represent only a small fraction of the stover biomass.

D. Effect of feedstock composition on process yield and economics

After completion of the NIR compositional analysis of the Wisconsin and Minnesota stover samples the tabulated data for each set were sorted by each chemical constituent to highlight the extreme values for each constituent. Five samples were selected from each set of samples on the basis of chemical variation in excess of two standard deviations from the mean value (i.e., 95% confidence interval) for the major constituents. Each sample was selected for a different constituent outlier pattern. One additional sample from each set was selected for its proximity to the mean for several of the constituents. The identity of the selected samples and their composition as determined by NIR is shown in Table 7.

Process models were run to determine the techno-economic effects of the various feedstock compositions and if the variations in yields change our conclusions regarding those effects. Each of the twelve selected stover compositions was investigated. The basis for this analysis is the current NREL design case reported in Aden, et al (2002), which has an overall process yield of 90 gal/dry ton and a minimum ethanol selling price (MESP) of $1.07/gal. That design case includes the following parameters, all of which are considered to be aggressive but achievable targets in a facility that starts operating in 2010.

- 90% yield of hemicellulose sugars to fermentable monomers in prehydrolysis;
- 90% conversion of cellulose to glucose by enzymatic saccharification;
- 95% conversion of glucose to ethanol and carbon dioxide in fermentation; and
- 85% conversion of xylose, mannose, galactose, and arabinose to ethanol and carbon dioxide in fermentation.

Corn stover compositions that were modeled for this analysis are shown in Table 7 and included a "design case" row to allow comparison between compositions used in this study and the design case report (Aden, 2002). Measured non-structural carbohydrates are modeled as extractives and are *not* considered to yield any ethanol. The sum of measured structural inorganics and soil are modeled as ash, and soluble solids are used to normalize the sum of all fractions to 1.0. Some of these assumptions may require revision in the future, as we learn more about the actual fates of extractives and soluble sugars in our process.

The above compositions resulted in ethanol yields ranging from 66.9 to 90.5 gal/dry ton feedstock, with the design case yield (89.8 gal/dry ton) close to the maximum. The MESPs (Minimum Ethanol Selling Price) range from $1.04/gal ethanol to $1.36/gal ethanol with the design case near the minimum ($1.07/gal). As expected, the yield is essentially linear as a function of carbohydrate composition in the feedstock. Figure 9 shows yield as a function of carbohydrate fraction and shows that the ethanol yield increases by 1.37 gal/dry ton for every 1% increase in total carbohydrate. Figure 10 shows MESP (Minimum Ethanol Selling Price) as a function of carbohydrate fraction. MESP follows a power function as shown in the figure, but using a straight line fit to this data, the minimum ethanol selling price is reduced by approximately 1.86 cents per gal for every 1% increase in total carbohydrate. That reduction is slightly smaller than the 2.1 cents/gal reported previously (Thomas, et al, 2001), and the difference is primarily caused by improved yields throughout the process. Scatter within the MESP figure is due to non-carbohydrates within the feedstock. For example, if two feedstocks have identical carbohydrate compositions but one has a higher fraction lignin than the other, the one with higher lignin will have a lower MESP because the overall energy available in the feedstock is higher so more steam and electricity are produced.

Table 7 – Fractional Composition of Corn Stovers Used in Techno-Economic Analysis

Sample Number	Extrac-tives	Structural glucan	Xylan	Galac-tan	Arabi-nan	Mannan	Lignin	Ash	Acetate	Protein	Soluble Solids
2798-002 MN	0.090	0.334	0.202	0.018	0.025	0.009	0.156	0.041	0.028	0.039	0.058
2798-012 MN	0.106	0.3113 [a]	0.1913	0.0159	0.0276	0.0072	0.1469	0.0542	0.0291	0.0451	0.0654
2891-004 MN	0.106	0.3245	0.1548 [a]	0.0127	0.02	0.0066	0.1498	0.068 [b]	0.0283	0.0463	0.083
2891-014 MN	0.077	0.359 [b]	0.2342 [b]	0.021	0.0367	0.0058	0.1609	0.0282	0.0388 [b]	0.0209 [a]	0.0175
2891-021 MN	0.0689	0.3524	0.2081	0.0176	0.027	0.0073	0.1766 [b]	0.038	0.0343	0.0346	0.0352
2892-027 MN	0.1126	0.3164	0.1718	0.0133	0.0244	0.0063	0.1362 [a]	0.07 [b]	0.0295	0.0425	0.077
2870-119 WI	0.0987	0.3509 [b]	0.1745	0.0055	0.0139	0.0121	0.1535	0.058	0.0271	0.0338	0.072
2893-025 WI	0.0736	0.3534 [b]	0.2022	0.0143	0.0254	0.0078	0.1703	0.045	0.0304 [b]	0.031	0.0466
2893-069 WI	0.0982	0.3225	0.2022	0.0133	0.0241	0.0109	0.1502	0.052	0.0247	0.0402	0.0617
2893-103 WI	0.1081	0.2963 [a]	0.1937	0.0164	0.0251	0.0091	0.1303	0.071	0.0207	0.0479 [b]	0.0814
2893-111 WI	0.1589 [b]	0.2911 [a]	0.1647 [a]	0.0058	0.009	0.014	0.1209 [a]	0.0743	0.009 [a]	0.0481 [b]	0.1042
2913-017 WI	0.0864	0.3316	0.2407 [b]	0.0179	0.0313	0.0107	0.1593	0.032 [a]	0.0264	0.0325	0.0312
Design Case	0.047	0.374	0.211	0.02	0.029	0.016	0.18	0.052	0.029	0.031	0.011

[a] greater than 2 standard deviations above the mean; [b] less than 2 standard deviations below the mean.

Figure 9 – Ethanol Yield vs. Structural Carbohydrate Content

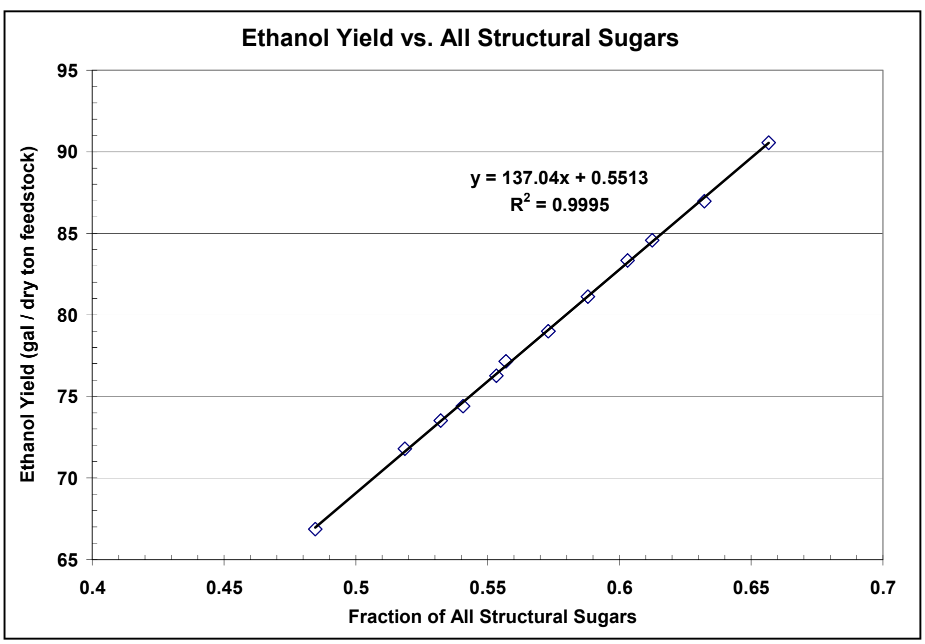
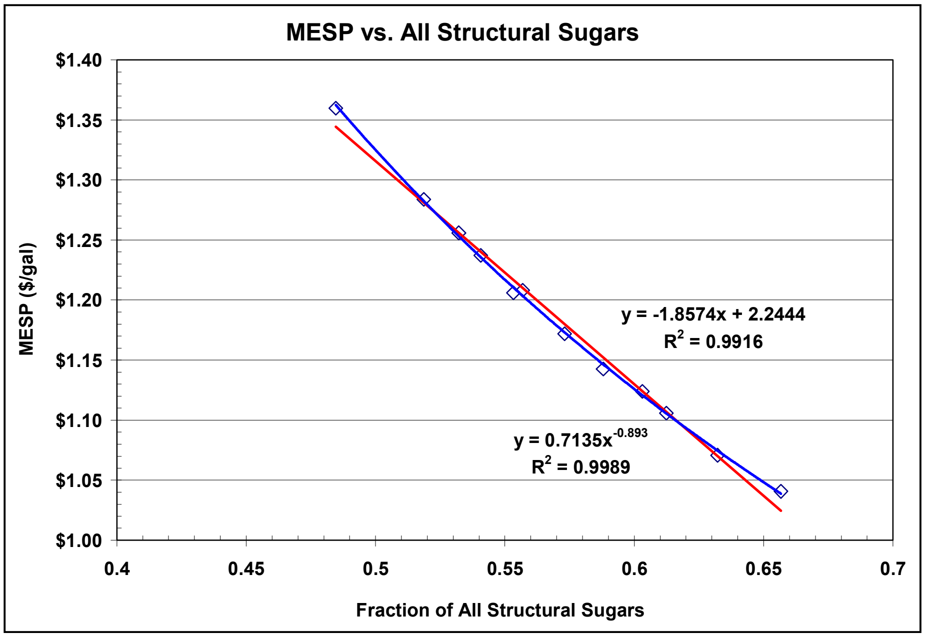


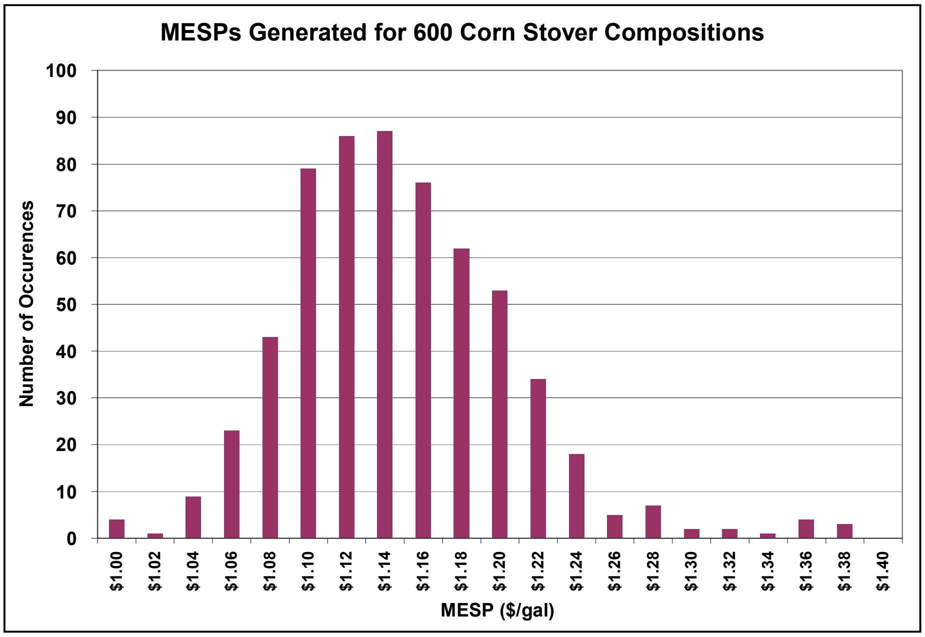
Figure 10 – MESP vs. Structural Carbohydrate Content



It is noteworthy that even though we selected samples for this analysis that represented both low and high carbohydrate contents that the design case model is so near the low end of the MESP range represented by these samples. In order to confirm this conclusion we calculated MESP for 600 of the 738 samples represented in our database to see if this was indeed the case. Figure 11 shows that data from the vast majority of samples produce an MESP well in excess of the design case $1.07 value. In fact, both the mean and median values for this set of samples are about $1.15/gal, with a standard deviation of $0.0584/gal. This means that about two-thirds of the samples generate an MESP between $1.09/gal and $1.21/gal. The choice of feedstock composition for the FY02 design case revision (Aden, et al, 2002) may have been overly optimistic.

The bottom line concerning stover quality is that since the carbohydrate content (i.e., total structural sugars) of corn stover can vary substantially from sample to sample, the yield of ethanol from a given sample under standard processing conditions and assumptions varies in a linear fashion. This can be thought of as the "ethanol potential" of a given feedstock. Process economics can therefore be substantially impacted, either positively or negatively, by the choice of feedstock lot.

Figure 11



III. Conclusions

The development of a robust rapid analysis method for determining the composition of raw corn stover was a prerequisite and invaluable tool that made possible the work described in this report. This work would never even have been proposed prior to the availability of the NIR method, as it would have been exceedingly expensive and time consuming to replicate this work using traditional wet chemical methods. This work provides evidence that the Stover5c NIR method is quite robust since it reliably predicted the composition of approximately 99% of the corn stover samples.

We have determined the composition of over 700 stover samples, representing a wide, but not comprehensive, cross-section of the genetic varieties commercially grown in the United States in 2001. We believe, but cannot prove, that this dataset describes the majority of the range of compositional variability among current hybrid corn varieties. More varieties grown in more diverse locations over a series of years should be surveyed to verify this.

The impact of the rather large range of compositions found in corn stover has a major impact on process economics, with MESP values ranging from $1.04 - $1.36/gal EtOH using the most recent design case model (Aden, et al, 2002), which produces an MESP for ethanol of $1.07/gal using a previously obtained average stover composition (see Table 7). The data from this work indicates that most stover would produce more expensive ethanol than this, suggesting that assumptions made about stover composition in the design case model are probably overly optimistic and should be revised.

As discussed previously, no samples in the population closely resemble the so-called "average stover composition" in Table 3, and this suggests that the concept of using an "average corn stover composition" in NREL's process modeling may not be justified. After all, does it really make sense to model a process based on a hypothetical feedstock composition that is either extremely rare, or non-existent? The Biomass Program should reconsider its choice of the feedstock basis used in process economic analyses. Either a real stover composition should be used, or composition should be expressed as ranges (+/- one standard deviation?) rather than specific values.

Our work indicates that levels of the major constituents of corn stover seem to vary according to a normal distribution with fairly wide ranges of values. This may not be true for some of the minor constituents.

Most pairs of constituents in stover show little or no evidence of positive or negative correlation with other constituents. In particular, variability in xylan shows no correlation with structural glucan, lignin, or acetyl levels. This general lack of correlation means that these constituents are relatively independent of one another. On the other hand, structural glucan does seem to correlate with both lignin (positively) and protein (negatively) content, and xylan content correlates negatively with structural inorganics.

We have confirmed that different anatomical fractions from a single variety have distinct compositions.

Among a set of five hybrids grown under four different conditions analysis of variance indicates that both genetic and environmental factors can strongly influence composition. Different constituents seem to be influenced by different patterns of factors, but this conclusion must be considered preliminary. Genetic and environmental factors may also interact to contribute to compositional variability.

IV. Suggestions for future work

<u>Near-term, defined end-point project</u>

Several hundred more stover samples are already on hand at NREL that are presumed to represent a greater range of genetic diversity than is present among the commercial hybrids surveyed in this study. This set of samples includes a high proportion of non-commercial varieties and includes some public inbred lines, open-pollinated varieties grown by farmers in the first half of the 20th century, landraces cultivated by Native Americans, wild accessions, and closely related species. From the perspective of trying to understand the breeding potential of corn in terms of stover composition, it would be worthwhile to see if a more exotic segment of the corn germplasm extends the range of compositional variability seen in this work. It is recommended that the existing stover samples be processed and analyzed for inclusion in the corn stover composition database. Depending on the results of this work, it may be worthwhile to explore corn germplasm collections in greater depth over a period of a few years. This would be done in conjunction with the USDA's National Plant Germplasm System.

The Program should work to establish and maintain strong relationships with corn breeders and agronomists at public and private institutions. These organizations are in a position to implement genetic strategies and altered cultivation and harvesting practices that can enhance the overall quality of corn stover (without sacrificing grain yield). The purpose of this stakeholder cultivation process is to convince seed companies, breeders and agronomists that biomass conversion is a business opportunity that they can contribute to developing.

<u>Longer-term critical R&D thrusts</u>

The variety of corn hybrids offered to farmers each year is constantly changing as new hybrids are released and older varieties are withdrawn from the market. The average market lifetime of a hybrid is probably on the order of 5 years, or so. The Program needs to understand if and how stover composition changes over time. It is recommended that this work be carried out in conjunction with state university-run grain yield trials in each maturity zone, as new hybrids are often entered into these trials to establish their competitiveness.

We do not yet understand the major causes of compositional variability in corn stover. While we have some evidence that both genetic and environmental factors play a role, it would be worthwhile to understand enough about this system to be able to manage compositional variability (if only partially).

The ability to manipulate stover composition to some extent through changes in agronomic practice or breeding could provide higher quality feedstocks for biomass conversion processes. A joint effort between DOE, USDA and selected agronomy departments at major universities is recommended to study this issue, to identify the major causes of stover variability, and to develop strategies to enhance stover quality for biomass conversion. This effort would leverage resources in ongoing efforts in the field of forage and silage quality that the USDA already funds at universities and its own labs.

Different stover materials sometimes exhibit different processing characteristics. This has mostly manifested itself as differences in pretreatment and enzymatic hydrolysis yields, but we currently do not understand what causes differential processing efficiency. It could be due to structural differences in cell walls between hybrids, suggesting that cell walls may be architecturally distinct. We currently have very few tools available that can help to distinguish such differences. It is recommended that the Program devote resources to developing tools that can distinguish differences in cell wall architecture (antibodies, cell wall degrading enzymes, microscopic techniques, NMR and other techniques). In addition, differences in processing efficiencies could be artifacts of the way in which stover is harvested, handled, stored or processed prior to introduction into the processing facility. In this case it would be useful to document the complete history of every bale of stover that comes in so that we can attempt to correlate processing behavior with sample history. As these two alternatives are not necessarily mutually exclusive, it is also recommended that the Program establish and maintain a database of feedstock materials that records all pertinent information about those materials, from planting through harvest and storage.

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